Predictive Dosimetry for Threshold Phototoxicity in Photodynamic Therapy on Normal Skin: Red Wavelengths Produce More Extensive Damage Than Blue at Equal Threshold Doses

Maria M. Tsoukas, Gloria C. Lin, Margaret S. Lee, R. Rox Anderson, and Nikiforos Kollias

Wellman Laboratories of Photomedicine, Department of Dermatology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, U.S.A.

The goal of this investigation was to establish methodology to determine and prevent phototoxic responses of normal skin to photodynamic therapy (PDT). The drug used was a second-generation photosensitizer, benzoporphyrin derivative monoacid ring A (BPD-MA). The dependence of skin phototoxicity on drug dose (0.5–2.0 mg/kg), fluence (1.2–390 J/cm^2), and wavelength (690 nm and 458 nm) was studied in the New Zealand albino rabbit in the first 5 h after injection. Skin responses were recorded for 2 wk after irradiation. Noninvasive measurements of drug fluorescence were made on unexposed skin sites during the first 5 h after drug injection. Immediate responses to PDT included erythema induced by 458 nm light and blanching induced by 690 nm light. Delayed reactions included edema on the day of exposure, purpura at 24 h, eschar by day 2 or 3, and scar by the end of follow-up. The threshold fluence for immediate responses correlated strongly with the threshold fluence for delayed reactions. The induction of threshold purpura on day 1 was a reliable index for skin phototoxicity that led to necrosis. The minimum purpura dose on day 1 after irradiation increased exponentially with the interval between drug injection and irradiation, independent of irradiation wavelength, for all drug doses. The action spectrum for threshold purpura mimics closely the absorption spectrum of BPD-MA. The *in vivo* drug fluorescence correlated with skin phototoxicity, thus allowing predictive dosimetry. This model system defines the safety limits for skin phototoxicity of PDT with BPD-MA. ***Key words: action spectrum/laser/benzoporphyrin derivative monoacid ring A (BPD-MA)/fluorescence spectroscopy. J Invest Dermatol 108:501–505, 1997***

Photodynamic therapy (PDT) has been developed over the past decade into a promising therapeutic modality for a wide range of solid tumors (Dougherty, 1987, 1993; Pass, 1993). PDT requires the administration of a tumor-localizing photosensitizing agent which, after exposure to visible light in the presence of oxygen, gives rise to highly reactive and cytotoxic species including singlet oxygen and free radicals. Both tumor and normal cells are susceptible to damage by these cytotoxic species (Dougherty, 1987). PDT efficacy is determined by a series of factors, such as the photosensitizer absorption spectrum, the wavelength of activating light, the presence of competing chromophores, the light penetration in tissue, the selective localization of the photosensitizer, and the biologic response of tissue to singlet oxygen.

Several PDT studies on cutaneous malignancies as well as benign skin lesions have been carried out after systemic administration of both first- and second-generation photosensitizers (Gomer, 1991; Lui *et al*, 1993). During clinical trials, drug doses, irradiation fluences, and fluence rates are being chosen experimentally, and PDT results have been variable (Gilson *et al*, 1988; Dougherty and Marcus, 1992). Major problems in PDT with first-generation porphyrins have included prolonged skin photosensitivity (Kessel *et al*, 1985) and limited penetration of light in tissue. This required improvements of therapeutic efficacy by the development of new photosensitizers and better determination of PDT dosimetry.

Benzoporphyrin derivative monoacid ring A (BPD-MA), a second-generation photosensitizer, is a semisynthetic porphyrin prepared by the reaction of protoporphyrin with dimethyl acetylenedicarboxylate (Richter *et al*, 1987). BPD-MA is a potent lipophilic photosensitizer *in vitro* (Richter *et al*, 1990b) and *in vivo* (Richter *et al*, 1991a). BPD-MA exhibits absorption maxima at 354, 418, 574, 626, and 688 nm in 50% methanol solutions. The 688 nm maximum shifts to 692 nm in aqueous solutions, with an extinction coefficient of 34,000 $M^{-1}cm^{-1}$ (Richter *et al*, 1990b). BPD-MA has proved *in vivo* to be a more efficient photosensitizer than hematoporphyrin compounds, with no latent skin phototoxicity (Richter *et al*, 1987, 1990a, 1991a,b; Lui *et al*, 1993).

This study was designed to investigate the responses of normal skin to PDT to establish safe limits for drug and light doses.

Manuscript received August 12, 1996; revised December 10, 1996; accepted for publication December 30, 1996.

Presented at The American Society for Photobiology Annual Meeting, 1994; and the 6th European Society for Photobiology, 1995.

Reprint requests to: Dr. Nikiforos Kollias, Wellman Laboratories of Photomedicine, Massachusetts General Hospital, Boston, MA 02114.

Abbreviations: BPD-MA, benzoporphyrin derivative monoacid ring A; MPuD, minimum purpura dose; PDT, photodynamic therapy.

0022-202X/97/$10.50

MATERIALS AND METHODS

Photosensitizer Liposomal BPD-MA was supplied by Quadra Logic Technologies Inc. (Vancouver, Canada). Drug was protected from light and maintained at 4°C until use. Liposomal BPD-MA for each experiment was reconstituted in sterile water (Abbott Laboratories, North Chicago, IL) at a concentration of 1.47 mg liposomal BPD per ml. The drug was administered at 0.5, 1, or 2 mg/kg body weight by bolus intravenous injection in the peripheral ear vein through a 24-gauge angiocatheter (Becton Dickinson Vascular Access, Sandy, UT), flushed with 2.5 ml of 5% dextrose in water (Baxter Healthcare Corp., Deerfield, IL).

Light Delivery Systems Light was produced at 690 nm with an argon ion pumped dye laser (CR 599 Dye Laser; Coherent, Palo Alto, CA) and at 458 nm with an argon ion laser (Innova 100; Coherent). The laser output was coupled into a microlens-tipped quartz fiber (Quadra Logic Technologies Inc.). The laser beam (3.6 cm in diameter) was adjusted perpendicular to the skin. The irradiance was 127 and 100 mW/cm^2 for 690 nm and 458 nm, respectively, and was measured with a power meter (Model 210; Coherent).

Animals The New Zealand albino rabbit was the animal model selected (Millbrook Breeding Farms, Amherst, MA). Animals weighed 2–3.5 kg each. The study design was approved by the Subcommittee on Research Animal Care at the Massachusetts General Hospital. The animals were anesthetized during the whole procedure by intramuscular injection of a mixture of ketamine (30 mg/kg) (Warner-Lambert Co., Morris Plains, NJ), xylazine (5 mg/kg) (American Regent Laboratories, Inc., Shirley, NY), and atropine (5 mg/kg) (Miles Inc., Shawnee Mission, KS). The animals were re-injected with 0.4 ml of the anesthetic mixture as necessary. Fur on the back was clipped (Model 5–01, size 40, Animal Clippers; Oster, Milwaukee, WI) and thereafter meticulously shaved ("Baxter" razors, Mansfield, MA) to obtain smooth skin appropriate for irradiations and optical measurements.

Normal Skin Phototoxicity *Versus* Time The reproducibility of skin responses to PDT and the duration of normal skin photosensitivity were investigated over the first 5 h after drug infusion. Animals (N = 13) were injected with 2 mg/kg BPD-MA and irradiated at hourly intervals (1–5 h after infusion) with 690 nm at a fixed fluence of 30 J/cm^2 and a fixed fluence rate of 127 mW/cm^2. Irradiations were duplicated at each time point on either side on the animal's back. The irradiated skin sites were 1.8 × 1.8 cm and were outlined by an adhesive-backed aluminized mylar template (MED 3044; DV Die Cutting, Inc., Danvers, MA). A site protected from light was defined as the control. The exposed skin sites were symmetrically located relative to the spine and were randomized to minimize systematic errors.

Action Spectrum The minimum dose to produce purpura at 24 h after exposure was determined at 458, 476, 488, 515, and 690 nm. Purpura was defined as a red-purple discoloration of the skin that could not be blanched with the application of pressure, unlike erythema that may be blanched. Animals (N = 2) were injected with 2 mg/kg BPD-MA and, starting at 1 h after drug injection, they were phototested at fluence increments of 100% of the previous dose, on four sites 1 cm in diameter at each wavelength. All tests were referenced to 690 nm. Thus, each test at different wavelengths was bracketed by two tests at 690 nm.

Dependence of the Minimum Purpura Dose (MPuD) on Drug Dose, Light Dose, and Wavelength The MPuD at 24 h after treatment was determined for exposures to light at 0–5 h after drug infusion with 690 and 458 nm radiation (N = 24). Six animals were treated at each wavelength and each drug dose, 0.5 mg/kg and 1.0 mg/kg. Irradiations were performed every hour from 0 to 5 h after drug injection. Light fluences ranged from 1.2 to 390 J/cm^2, delivered at 127 and 100 mW/cm^2 for irradiations at 690 and 458 nm, respectively. At each time point, four skin sites 1 cm in diameter were simultaneously exposed to a range of fluences at increments of 40% of the previous dose. Unirradiated control sites were also marked on either side of the animal's back.

Clinical Evaluation of Skin Reactions The exposed and control sites were observed before and after irradiation, at 30-min intervals for 8 h, and at 1, 2, 5, and 15 d after exposure. Skin reactions were scored on the scale given below. Blanching, erythema, edema, and purpura were individually graded as follows: −, no reaction; ±, minimal response without filling the entire exposed area; 1+, response of higher intensity over the entire exposed area, with clear margins; 2+, more pronounced response (for edema, the response extended beyond the borders of the exposed site); and 3+, marked response extending more than 0.5 cm from the margin of the exposed site. The appearances of scale, scab, and scar were also recorded.

***In Vivo* Fluorescence Measurements** Because BPD-MA has a significant fluorescence quantum efficiency and a significant extinction coefficient (55,000 at 430 nm and 30,000 at 690 nm), fluorescence spectra were obtained *in vivo* from an unexposed control skin site and from all treated sites before and after exposure. Fluorescence spectra were collected with a fluorescence spectrophotometer (Fluoromax; SPEX Industries, Edison, NJ) equipped with a fiber bundle assembly so that *in vivo* fluorescence spectra could be obtained from any site on the animal's back. During the measurements, the joined end of the fiberoptic assembly was brought in contact with the skin site under study. *In vivo* excitation spectra were obtained for emission at 690 nm on animals injected with 2 mg/kg BPD-MA. The 430-nm excitation maximum obtained *in vitro* from BPD-MA solutions in fetal bovine serum was found to shift to 456 ± 2 nm *in vivo* because of the competing absorption of hemoglobin at 430 nm. Emission spectra were obtained with excitation at both maxima of the drug absorption at 450 nm and 690 nm.

Emission spectra were obtained from an unexposed control site with excitation at both 450 nm and 690 nm because of the difference in tissue penetration of these two wavelengths. When exciting at 450 nm, the emission spectrum was recorded from 550 to 800 nm; when exciting at 690 nm, the emission spectrum was recorded from 700 to 800 nm. The *in vivo* fluorescence signal was always corrected for the normal skin fluorescence obtained before drug infusion. The calculated fluorescence signal presented here is the area under the fluorescence emission curve for either 450 or 690 nm.

There are two reasons why the fluorescence intensity excited at 450 nm may be different from that excited at 690 nm. The first involves the fluorescence efficiency of the drug and the second the distribution of the drug in the skin. To estimate changes in fluorescence intensity due to the fluorescence efficiency of the drug at different wavelengths, we calibrated with homogeneous phantoms. Scattering phantoms were constructed consisting of suspensions of microspheres 1.06 μm in diameter (Duke Scientific, Raleigh, NC) at a concentration of 2% solids, which simulates human dermis. Serial dilutions of BPD-MA were made in the scattering phantoms, and emission spectra were collected for excitation first with 450 nm and then with 690 nm. The suspension was shaken between repeated measurements to minimize precipitation of the microspheres.

RESULTS

Normal Skin Phototoxicity *Versus* Time, at Constant Drug Dose and Constant Light Dose The cutaneous photobiologic responses to PDT with BPD-MA at 2 mg/kg with a fluence of 30 J/cm^2 at 127 mW/cm^2, 690 nm light, proved reproducible when treatment was delivered in the first 5 h after drug administration. The responses were most severe on the sites irradiated at the early time points after injection and gradually decreased in intensity and duration with time. Skin blanching without edema was clearly visible immediately after exposure on all skin sites treated in the first 4 h after drug injection. The blanching disappeared with time and was followed by a prominent edema on the sites treated at 1–3 h after drug injection. Severe edema responses included 1- to 2-mm purpuric margins. The sites that were exposed 4 h after injection showed mild responses, and the sites exposed at 5 h after injection demonstrated minimal if any reactions **(Fig 1)**.

The skin sites that were exposed 1–4 h after drug injection showed edema and purple-red purpura 1 d after treatment. The purpura induced on the sites treated 1–2 h after injection appeared deeper and were bluish-white; on the sites treated 3–4 h after injection, the purpura was more red in appearance. The purpura induced in the sites exposed 4 h after injection often showed a superficial meshwork of thrombosed vessels. The purpuric reactions became deep gray-brown by day 2 after treatment, with arrest of hair regrowth. The ring of erythema that surrounded the edema response at day 1 developed into purpura. Eschar started forming on day 2 and progressed into a concave, dark purple-brown scab approximately half the size of the originally exposed site. When the eschar was removed 15 d after exposure on the threshold purpura doses, there was a flat atrophic scar that appeared pink-yellow and glossy. Sites that were treated 5 h after drug injection showed slight edema on days 1 and 2 after exposure, accompanied at times by slight purpura or erythema, and returned to normal shortly thereafter.

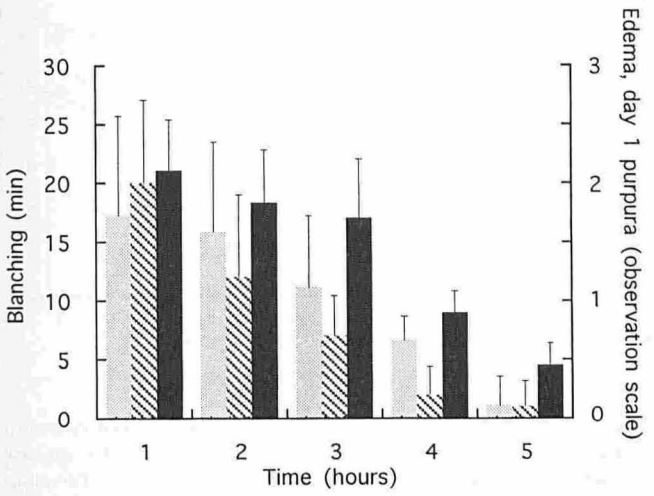

Figure 1. Skin responses to PDT with BPD-MA decrease rapidly with time after injection. The early response of blanching shows good correlation with the delayed reactions of edema (4 h) and purpura (24 h). The intensity of the blanching reaction (□) is expressed in terms of its duration (min). Edema (▨) and purpura (■) were graded on a 0–3 scale. Phototoxicity was induced with 30 J/cm^2 (127 mW/cm^2) of 690 nm radiation after injection of 2 mg/kg of drug. *Error bars*: ±SD, N = 13.

Action Spectrum for MPuD Follows Closely the Absorption Spectrum of the Drug The threshold responses at all wavelengths tested were comparable. At 690 nm and with doses higher than the MPuD, the skin responses extended beyond the limit of exposure in a concentration-dependent manner. At the shorter wavelengths, the purpura was always confined within the limits of exposure. The threshold fluence for purpura was minimum at 458 and 690 nm. The results are plotted as the reciprocal of the MPuD and correlate well with the absorption spectrum of the drug **(Fig 2)**.

The MPuD Is Strongly Dependent on the Injected Drug Dose and the Light Dose; It Is Independent of the Wavelength of Irradiation The MPuD was the same as the fluence to produce skin necrosis (scab) on day 2–3 and scar on day 15 **(Fig 3*a*)**. The MPuD increased quickly with time after injection (exponentially) and was found to be wavelength independent at both drug doses tested (0.5 and 1.0 mg/kg) **(Fig 3*b*)**. It is important to note that although the MPuD was the same at the two wavelengths tested, the photobiologic responses before necrosis and the extent and depth of damage were significantly different. The MPuD, on the other hand, showed a stronger than linear dependence on the injected drug dose. When the drug dose was doubled, the MPuD decreased by a factor of 4.

Effects of 690 nm Light Exposures at MPuD with 690 nm radiation produced immediate blanching, which lasted for a few minutes (with no edema). At 2.5 h after exposure, the blanching was followed by 1+ edema, which developed into purpura that completely filled the exposed site 24 h after exposure **(Fig 4*a*)**. Superthreshold exposures produced a concave scab, the diameter of which increased with increasing dose.

Effects of 458 nm Light The overall responses of the skin to 458 nm radiation were milder than those produced by 690 nm radiation. At MPuD, a mild diffuse erythema (±) appeared immediately after exposure, which was followed by edema (1+) at 6–7 h **(Fig 4*b*)**. At 24 h after exposure, the site had purpura (reddish-light brown). Skin sites irradiated at a dose 40% lower than the MPuD showed a superficial light brownish discoloration that resolved. Superthreshold exposures produced a flat scab, the diameter of which remained constant with increasing dose. The severity of damage that followed the 24-h purpura was more intense for 690 nm than for 458 nm radiation.

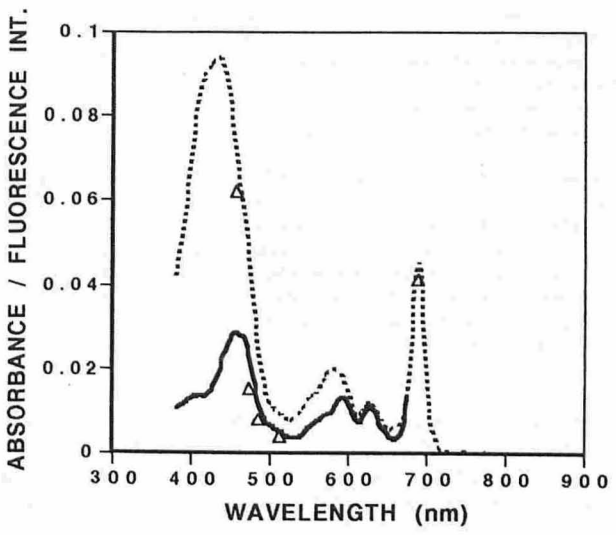

Figure 2. The absorption spectrum of BPD-MA *in vitro* and the fluorescence excitation spectrum of BPD-MA *in vivo* for emission at 690 nm have very similar features. The differences in the two spectra are due to the absorption of hemoglobin. The two spectra are normalized at 620 nm, as there is little contribution by hemoglobin at that point. The *in vivo* excitation spectrum *(solid line)* of BPD-MA indicates absorption maxima at 456 ± 2 and 690 nm; these wavelengths were therefore selected for treatments. The reciprocal of the fluence to produce threshold purpura data on day 1 (△) is also plotted along with the absorption spectrum *(dotted line)*. Note that the action spectrum follows the general features of the absorption spectrum. INT., intensity. (N = 2).

Noninvasive Fluorescence Closely Follows Phototoxicity The excitation fluorescence spectrum of BPD-MA *in vivo* was obtained from animals injected with 2 mg/kg **(Fig 2)**. It proved somewhat different from the absorption spectrum because of attenuation of the excitation light by hemoglobin. The native fluorescence obtained from the animal's skin before drug infusion with excitation at 450 or 690 nm proved stable and reproducible. Spectra obtained after infusion were corrected for native fluorescence of the skin and yielded spectral features characteristic of the fluorescence of BPD-MA. The fluorescence emission maximum and band width remained constant with time after infusion. For exposures with a single fluence of 30 J/cm^2 in the first 5 h after injection, a time-dependent decrease of fluorescence emission signal was found, which corresponded to decreased phototoxicity of the animals' skin with time. In the experiments to determine the MPuD, fluorescence emission spectra were collected at 0–5 h after infusion from a control site on each animal.

The integrated fluorescence obtained from the homogeneous phantoms excited at 450 nm was 11.1 times greater than the corresponding fluorescence excited at 690 nm. Once this correction has been applied to the integrated fluorescence obtained *in vivo*, the two signals may be displayed on the same scale **(Fig 5*a,b*)**. Fluorescence emission upon excitation at 450 nm showed a substantially slower decrease with time after injection compared with the fluorescence signal with excitation at 690 nm. The integrated fluorescence intensity increased linearly with drug dose.

DISCUSSION

Photobiologic skin responses to lipophilic photosensitizers have been investigated previously (Moore *et al*, 1986; Razum *et al*, 1987; Bellnier and Dougherty, 1989; Henderson and Dougherty, 1992). The efficacy of PDT with hematoporphyrin compounds has been evaluated on tumor models (van Gemert *et al*, 1985; Dougherty, 1987; Fingar and Henderson, 1987), including the wavelength dependence (Star *et al*, 1990). In this study, a systematic approach was used to determine the dosimetry of photodynamic drugs on

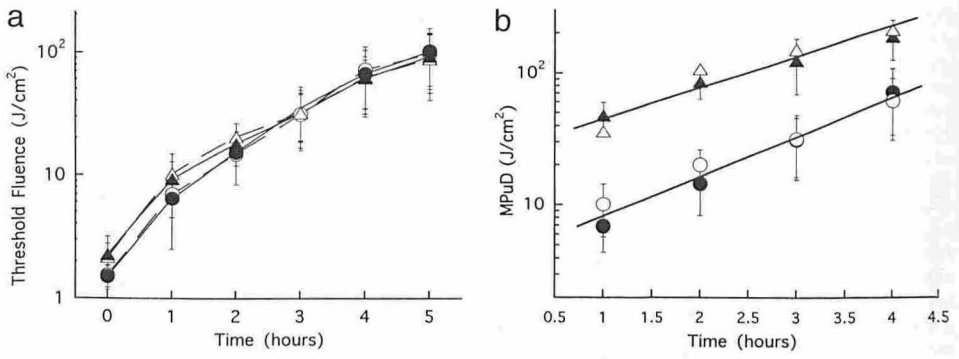

Figure 3. The minimum purpura dose is independent of wavelength for all time points and for all drug doses. *(a)* Animals were injected with 1 mg/kg BPD-MA and were treated with 690 nm *(circles)* and 458 nm *(triangles)* light at 127 and 100 mW/cm^2, respectively, from 0 to 5 h after injection. *Solid markers,* threshold inducing necrosis observed by day 15; *open markers,* threshold for day 1 purpura. *b)* Animals were treated with 1 mg/kg *(circles)* and 0.5 mg/kg *(triangles)* BPD-MA with either 690 nm light *(solid markers)* or 458 nm light *(open markers). Lines* represent the best fits through the experimental points. *Error bars*: ±SD, N = 6.

normal skin. BPD-MA, a potent lipophilic second-generation photosensitizer, was used to determine the acceptable limits of drug and light to avoid phototoxicity in normal skin. We determined that skin responses of this animal model were stable, reproducible, and similar in expression to those of normal human skin (Lui *et al*, 1993). In human skin, the immediate response included a wheal and flare reaction; the flare subsided with time whereas the edema remained for several days. Purpura that appeared by day 2 frequently resulted in normal skin necrosis. The drug doses investigated and reported here were selected so that the light doses necessary for the formation of purpura were not less than 1 J/cm^2 immediately after injection (10 s exposure) and not more than 250 J/cm^2 at 5 h after injection (45 min exposure).

Constant Drug Dose and Constant Light Dose Allow Determination of the Range of Photobiologic Skin Reactions Skin phototoxicity decreased rapidly with time after drug injection at all drug doses. The photobiologic responses of immediate blanching, delayed edema, and gross sparing of the epidermis on the day of exposure, as well as purpura on day 1, indicate that the PDT action of this drug takes place in the skin vasculature. The rapid decrease of skin phototoxicity after bolus injection of the drug (even at high doses of 2 mg/kg), due to a constant irradiation fluence, also reinforces the dependence of the PDT effect on the circulating drug concentration.

Hemoglobin in the skin appears to play an important role in determining the effective absorption of the drug in tissue. This became clear when we compared the excitation fluorescence spectrum of the drug in the skin with the absorption spectrum of the drug in fetal bovine serum **(Fig 2)**. The ratio of these spectra yielded the spectrum of hemoglobin. The action spectrum for the MPuD was found to track the absorption spectrum (Waterfield *et al*, 1994).

The Treatment Wavelength Selects the Extent of Damage But Not the Light Dose to Produce Toxicity The two wavelengths investigated, blue and red (458 and 690 nm), induced phototoxicity at similar threshold fluences for both drug doses explored. The extent of necrosis (beyond the limits of exposure) produced with superthreshold fluences at 690 nm light appeared more profound than that produced with 458 nm light. In this sense, the biologic responses parallel the depth of penetration of the two wavelengths. It is also interesting that the early vascular responses induced by these two wavelengths were diametrically opposite. Immediately after exposure, the red light induced blanching, whereas the blue light induced erythema. Although the attenuation of the incident light in skin at these two wavelengths is different by a factor of 4 (Anderson and Parrish, 1981), the threshold fluence for purpura and eventual skin necrosis proved to be the same. The MPuD proved a useful clinical end point for phototoxicity, however. Because the early responses (blanching with 690 nm and erythema with 458 nm) correlated well with late responses, they could be used to predict the threshold fluence for purpura. One can envision performing a phototest on a small site (e.g., 4 mm) to determine the blanching threshold before proceeding with treatment of a large area.

The Drug Fluorescence Measured *In Vivo* Is Closely Related to Phototoxicity Results showed that the 450-nm excited fluorescence did not change very much with time after drug injection, whereas the 690-nm excited fluorescence showed a significant decrease with time. Skin levels of BPD-MA remain essentially constant over the first 5 h after injection, while the plasma level changes as an exponential function of time (Richter *et al*, 1994). Radiation of wavelength 450 nm penetrates 4 times less than 690-nm radiation (Anderson and Parrish, 1981); furthermore, 450-nm radiation is attenuated by hemoglobin, resulting in little fluorescence from the circulating drug. In contrast, fluorescence excited with 690 nm radiation would probe both the epidermis and dermis, which would include the plasma drug concentration, as this wavelength is not attenuated by the absorption of hemoglobin. Therefore, by subtracting the tissue contribution (450-nm excited fluorescence at 5 h after injection) from the signal obtained from tissue plus blood (690-nm excited fluorescence), we should obtain a signal that is associated primarily with the plasma contribution. Then the corrected fluorescence at each time point can be plotted *versus* the corresponding threshold dose for purpura formation for both drug doses used (0.5 and 1 mg/kg) **(Fig 6)**. We can thus

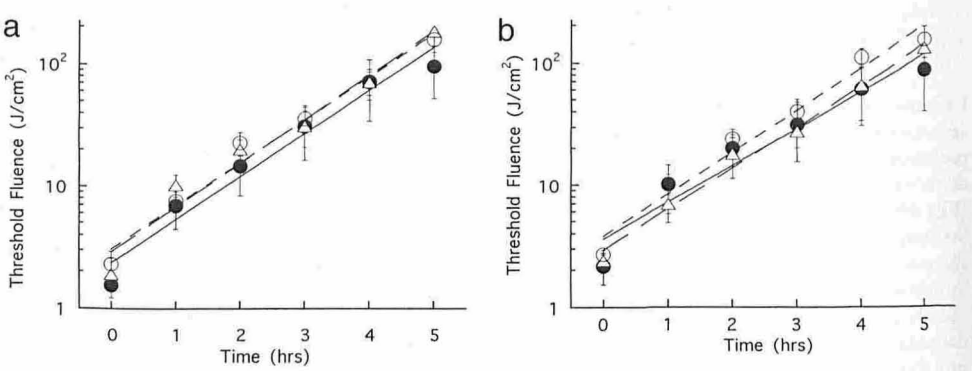

Figure 4. The early and late phototoxic reactions to PDT correlate strongly for both red light and blue light. *(a)* The early skin reaction (blanching, *triangles*) and late skin reactions (4-h edema, ○; 24-h purpura, ●) to PDT with BPD-MA correlate closely for exposures to 690 nm light at 127 mW/cm^2. *Lines* represent the best fits through the experimental points. *(b)* The early skin reaction (erythema, *triangles*) and late skin reactions (7-h edema, ○; 24-h purpura, ●) to PDT with BPD-MA correlate closely for exposures to 458 nm light. *Lines* represent the best fits through the experimental points. *Error bars*: ±SD, N = 6.

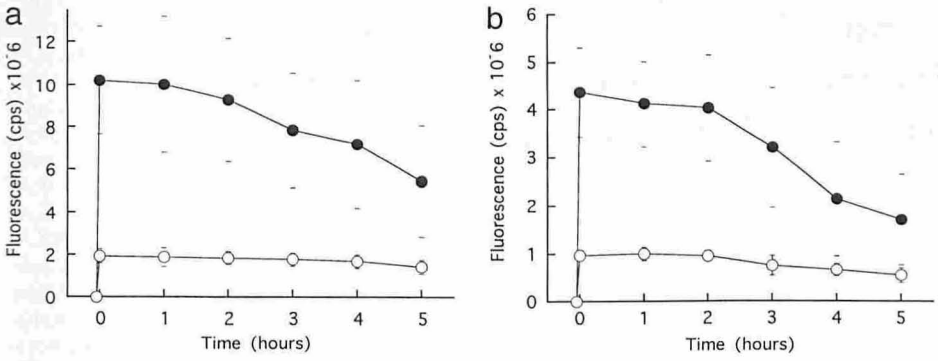

Figure 5. The *in vivo* drug fluorescence excited with 690 nm decreases with time after drug injection, whereas that excited at 450 nm remains constant. The integrated *in vivo* drug fluorescence excited at 690 nm (●) at 1 mg/kg *(a)* and 0.5 mg/kg *(b)* shows a decrease over time, compared with the signal obtained when excited at 450 nm (○). The experimental points represent areas under the fluorescence emission curve corrected for the skin fluorescence before drug injection and were normalized for fluorescence efficiency at 450 and 690 nm. *Error bars*: ±SD, N = 6.

define a region of safety in **Fig 6** as the part below the curve and a region of induction of skin necrosis above the curve. A range of safe fluence values is defined based on *in vivo* fluorescence measurements at all time points and for both drug doses.

For effective dosimetry with PDT on normal skin, we must establish (i) the phototoxic response of skin to the drug under a constant light and drug regimen, scoring individually all the skin reactions; (ii) the threshold dose of light at different wavelengths (where the drug absorbs significantly) to produce irreparable damage; (iii) whether noninvasive optical means exist that allow the determination of safe dosimetry for normal skin; and (iv) translation of results obtained from other species to the human case. Once the parameters that spare normal skin have been established, one can proceed with confidence to treat skin diseases. BPD-MA has been characterized, and the extent of damage can be adequately described by the threshold fluence to produce purpura (MPuD). The threshold phototoxic fluence increases with time almost exponentially, independent of the wavelength of exposure (red or blue). The early expression of phototoxicity and severity of damage are different for blue and red light. Fluorescence measured *in vivo* can be used to predict correctly the safe limits of light dose.

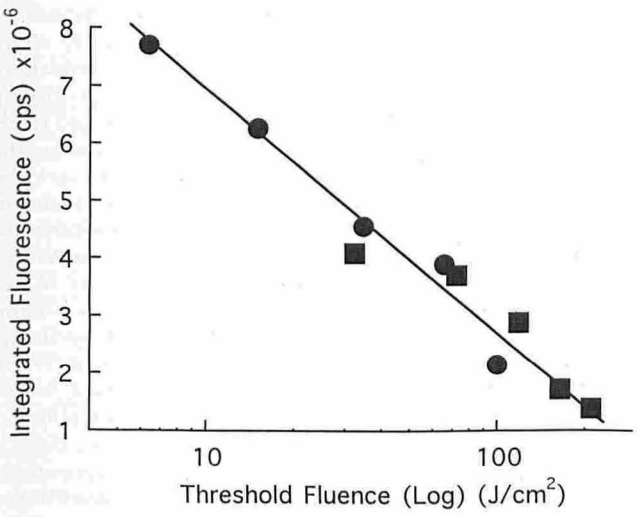

Figure 6. The corrected fluorescence correlates with the MPuD and allows the prediction of safety levels. The integrated fluorescence excited at 690 nm, normalized for fluorescence efficiency and corrected for the tissue component by subtracting the fluorescence excited at 450 nm at 5 h after drug injection, at both drug doses of 0.5 mg/kg (■) and 1.0 mg/kg (●), correlates with the MPuD. *Line* represents the best fit to the experimental points and defines the limit of phototoxicity. Doses that correspond to points that lie between the experimental line and the horizontal axis are safe, whereas doses above the line would produce necrosis. (N = 6).

We are grateful to our colleagues P. Dwyer, W. Farinelli, R. Gillies, D. McAuliffe, and J. Wimberly of Wellman Labs and M. Stanfield of QLT for their help and support. We thank Anna Richter for her suggestions and for a critical reading of the manuscript. This work was partially supported by Quadra Logic Technologies Inc.

REFERENCES

Anderson RR, Parrish J: The optics of human skin. *J Invest Dermatol* 77:13–19, 1981

Bellnier DA, Dougherty TJ: The time course of cutaneous porphyrin photosensitization in the murine ear. *Photochem Photobiol* 49:369–372, 1989

Dougherty TJ: Photodynamic therapy. *Photochem Photobiol* 58:895–900, 1993

Dougherty TJ: Photosensitizers: therapy and detection of malignant tumors. *Photochem Photobiol* 45:879–889, 1987

Dougherty TJ, Marcus SL: Photodynamic therapy. *Eur J Cancer* 28A:1734–1742, 1992

Fingar VH, Henderson BW: Drug and light dose dependence of photodynamic therapy: a study of tumor and normal tissue response. *Photochem Photobiol* 46:837–841, 1987

Gilson D, Ash D, Driver I, Feather JW, Brown S: Therapeutic ratio of photodynamic therapy in the treatment of superficial tumors of skin and subcutaneous tissues in man. *Br J Cancer* 58:665–667, 1988

Gomer CJ: Preclinical examination of first and second generation photosensitizers used in photodynamic therapy. *Photochem Photobiol* 54:1093–1107, 1991

Henderson BW, Dougherty TJ: How does photodynamic therapy work? *Photochem Photobiol* 55:145–157, 1992

Kessel D, Chang CK, Musselman B: Chemical biologic and biophysical studies on hematoporphyrin derivative. In: Kessel D (ed.). *Methods in Porphyrin Photosensitization*. Plenum Press, New York, 1985, pp 213–227

Lui H, Hruza L, Kollias N, Wimberly J, Anderson RR: Photodynamic therapy of malignant skin tumors with benzoporphyrin derivative-monoacid ring A (BPD-MA): preliminary observations. *Proc SPIE* 1876:147–151, 1993

Moore JV, Keene JP, Land EJ: Dose-response relationships for photodynamic injury to murine skin. *Br J Radiol* 59:257–261, 1986

Pass HI: Photodynamic therapy in oncology: mechanisms and clinical use. *J Natl Cancer Inst* 85:443–456, 1993

Razum N, Balchum OJ, Profio AE, Carstens F: Skin photosensitivity: duration and intensity following intravenous hematoporphyrin derivatives, Hpd and DHE. *Photochem Photobiol* 46:925–928, 1987

Richter AM, Cerruti-Sola S, Sternberg ED, Dolphin D, Levy JG: Biodistribution of tritiated benzoporphyrin derivative (^{3}H-BPD-MA), a new potent photosensitizer, in normal and tumor bearing mice. *J Photochem Photobiol B* 5:231–244, 1990a

Richter AM, Jain AK, Obochi M, Meadows H, Canaan AJ, Levy JG: Activation of benzoporphyrin derivative in the circulation of mice without skin photosensitivity. *Photochem Photobiol* 59:350–355, 1994

Richter AM, Kelly B, Chow J, Liu DJ, Towers GHN, Dolphin D, Levy JG: Preliminary studies on a more effective phototoxic agent than hematoporphyrin. *J Natl Cancer Inst* 79:1327–1332, 1987

Richter AM, Waterfield E, Jain AK, Allison B, Sternberg ED, Dolphin D, Levy JG: Photosensitizing potency of structural analogues of benzoporphyrin derivative (BPD) in a mouse tumor model. *Br J Cancer* 63:87–93, 1991a

Richter AM, Waterfield E, Jain AK, Sternberg ED, Dolphin D, Levy JG: *In vitro* evaluation of phototoxic properties of four structurally related benzoporphyrin derivatives. *Photochem Photobiol* 52:495–500, 1990b

Richter AM, Yip S, Waterfield E, Logan PM, Slonecker CE, Levy JG: Mouse skin photosensitization with benzoporphyrin derivatives and photofrin: macroscopic and microscopic evaluation. *Photochem Photobiol* 53:281–286, 1991b

Star W, Versteeg J, van Putten W, Marijnissen H: Wavelength dependence of hematoporphyrin derivative photodynamic treatment effects on rat ears. *Photochem Photobiol* 52:547–554, 1990

van Gemert JC, Berenbaum MC, Gijsbers GHM: Wavelength and light dose dependence in tumor phototherapy with haematoporphyrin derivative. *Br J Cancer* 52:43–49, 1985

Waterfield EM, Renke ME, Smits CB, Gervais MD, Bower RD, Stonefield MS, Levy JG: Wavelength dependent effects of benzoporphyrin derivative monoacid ring-A *in vivo* and *in vitro*. *Photochem Photobiol* 60:383–387, 1994